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Root-Associated Nitrogen Fixation in Finger Millet

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Summary

This paper presents the results of investigations carried out on some aspects of root-associated nitrogen fixation in finger millet (Eleucine coracana *Gaertn). Soil-root nitrogenase assay of 53 finger millet cultivars belonging to four maturity groups indicated (1) low levels of nitrogenase activity, (2) high plant-to-plant variability, (3) high variability between cultivars, (4) higher activity in hybrids over pure lines, (5) higher activity in medium-maturity group over early- or late-maturity groups, and (6) no apparent relationship between nitrogenase activity and dry-matter production. Nitrogenase activity of finger millet soil-root system was further affected by plant age, soil moisture, and diurnal changes. All cultivars had highest diazotroph counts during the reproductive stage in root-adhering soil, root wash, and unsterilized root macerate. The root-associated nitrogen fixers were species of* Pseudomonas *and* Enterobacter. *Inoculation of finger millet cultivar Indaf 7, with NBRE and SRI diazotroph cultures, increased above-ground yields and N uptake over the control in the greenhouse, but not in the field. Nitrogen-balance study with inoculation and three levels of applied nitrogen showed a net gain equivalent to 6.3-9.4 kg N* ha^{-1} *from inoculation.*

Introduction

Finger millet (*Eleusine coracana* Gaertn) is an important food crop in the semi-arid tropics (SAT), particularly in India and Eastern Africa. The crop ranks fourth in area (5 million ha) and production (4.5 million t) among millets in the world. India accounts for 45% of the world production.

Contributions of nonsymbiotic nitrogen fixation ociated with finger millet and other millets, though felt to be substantial, have been wide ranging (20-148 kg N ha^{-1}) due to different methodologies used by different workers (Moore 1963, Jenkinson 1977, Dart and Wani 1982). Studies were conducted at the University of Agricultural Sciences, Bangalore, on (1) root-associated nitrogenase activity of 53 finger millet cultivars, (2) occurrence of diazotrophs, (3) inoculation response, (4) factors affecting nitrogen fixation, and (5) nitrogen balance.

Screening Germplasm

Fifty-three cultivars representing both pure-line selections and hybrids in four maturity groups were screened for their ability to stimulate nitrogenase activity (C_2H_2 reduction) in the rhizosphere at the flowering and postflowering growth stages (Table 1). Unplanted soil and finger millet cultivars Indaf 5 were included as checks in each group. The plants were grown in polythene bags filled with 3.3 kg soil. The soil surface in each bag was covered with a 5-cm thick gravel, and the bags were kept immersed in a sand bed to avoid algal growth at the soil surface and

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at the interface of soil and bag. A basal dose of 50 kg each of N, P_2O_5, and K_2O ha^{-1} equivalent was applied through ammonium sulfate, superphosphate, and potassium chloride respectively. Plants were watered as required with tap water and one day before assay the moisture content of the bags was adjusted to 70-80% water-holding capacity (WHC). For nitrogenase assay, the method followed by Wani et al. (1983) was used.

The general level of nitrogenase activity associated with 53 finger millet cultivars was low, and the group means ranged from 2 to 267 nmoles C_2H_4 h^{-1} bag^{-1} or 1 to 59 nmoles h^{-1} g^{-1} root mass (Table 1). Ranking of cultivars based on root activity differed when expressed as g^{-1} root than on bag^{-1} basis. The mean nitrogenase activity of 0.2-13 nmoles h^{-1} bag^{-1} in the unplanted soil suggested stimulation of nitrogenase activity by the plant. Increased nitrogenase activity in Assay II over Assay I in maturity groups I and II indicated higher activity during post-flowering growth stages. The maximum nitrogenase activity of individual plants in this study was 488 nmoles C_2H_4 h^{-1} bag^{-1}. This is much lower than the activity of 1064 nmoles C_2H_4 h^{-1} $core^{-1}$ recorded for finger millet cultivar PR 202 at ICRISAT Center (ICRISAT 1978) and 374 nmoles C_2H_4 h^{-1} g^{-1} dry mass by Dommergues et al. (1973).

Within a cultivar, plant-to-plant variation in nitrogenase activity from 10 to 488 nmoles C_2H_4 h^{-1} bag^{-1} in cultivar TNAU 169 and 5-359 nmoles C_2H_4 h^{-1} bag^{-1} in cultivar Indaf 5 was observed. Such variations have also been recorded in sorghum and pearl millet (Wani et al. 1983).

Of the 53 cultivars tested, the activity was more

Table 1. Nitrogenase activity (nmoles C_2H_4 h^{-1})[1] of soil-root system of finger millet cultivars during two assays.[1]

Maturity group (Days to 50% flowering)	Cultivar	Assay I			Assay II		
			Range/Cultivar[2]			Range/Cultivar[2]	
		DAS[3]	bag^{-1}	g^{-1} root	DAS	bag^{-1}	g^{-1} root
I (56-62)	VL 114,VL 115,VL 116 VL 118,VL 110, PES 83-2,PES 400 RAU 3,RAU 7,RAU 5,RAU 2, REC 45-1,REC 13-1	55	12-70 REC 13-1 REC 45-1 (38)	2-30 REC 13-1 VL 115	62	10-138 REC 45-1 PES 83-2 (68)	4-46 REC 45-1 PES 83-2
II (63-70)	VL 117,Indaf 9,HR 374, PR 177,PR 1103, PR 1091,PES 176, TNAU 169,TNAU 167, TNAU 193,TNAU 256, TNAU 160,TNAU 294	70	2-30 TNAU 160 TNAU 169 (13)	1-11 TNAU 160 TNAU 169	92	56-267 VL 117 TNAU 169 (131)	8-59 PR 1103 PR 1091
III (71-78)	HR 919,HR 154,HR 2823 ROH 2,HR 1541, Purna,PR 230,PR 202, B 7-7-43,PES 110,JNR 981-1 JNR 852,JNR 1008		ND[4]	ND	94	21-166 PR 230 B 7-7-43 (85)	3-24 PR 230 B 7-7-43
IV (79-89)	Indaf 1,Indaf 3,VR 550, Indaf 7,Indaf 8, Indaf 10,Indaf 11,VR 530 KM 13,IE 1012,U 6,U 10 (51) A 104		ND	ND	99	19-12 Indaf 8 IE 1012	4-32 Indaf 8 Indaf 10

1. Mean of 4 replications. Indaf 5 and unplanted soil were used as checks. Nitrogenase activity of unplanted control ranged from 0.2 to 13 nm C_2H_4 bag^{-1}.
2. Mean nitrogenase activity.
3. DAS = Days after sowing.
4. ND = Not determined.

than 300 nmole C_2H_4 h^{-1} bag^{-1} in 12 cultivars, between 200 to 300 in 8 cultivars, between 100 to 200 nmol in 20 cultivars, and less than 100 in 13 cultivars. Such variability for nitrogenase activity amongst finger millet cultivars has also been observed earlier (ICRISAT 1978). The top five cultivars, based on maximum activity, were TNAU 169, TNAU 256, Purna, IE 1012, and Indaf 9, in that order. Least activity was found in RAU 7. Generally, hybrid selections showed higher nitrogenase activity than pure-line selections (Table 2).

The medium-maturity cultivars (groups II and III) had higher nitrogenase activity than either early- (group I) or late-maturity cultivars (group IV). Mishra et al. (1980) had also found that medium-maturity cultivars were more productive than early or late types, based on their screening of 400 finger millet cultivars.

Seasonal Variation in Diazotroph Counts and Nitrogenase Activity

Plants of cultivar Indaf 5 were similarly grown in polythene bags, except that 2.75 kg soil was used in this experiment. The plants were inoculated with 50 mL rhizosphere-soil extract. This extract was prepared by mixing 1 kg rhizospheric soil collected from the field with 4 L N-free sucrose medium, incubated for 42 h at room temperature, filtered through a cheese cloth, and finally made up to 10 L using water. An unplanted treatment was also included. Six randomly selected plants were assayed for nitrogenase at 22, 32, 44, 59, 68, 81, 105, and 129 days after sowing (DAS), following the method mentioned earlier.

Nitrogenase activity increased from 22 DAS (9 nmol C_2H_4 h^{-1} bag^{-1}) to peak at 68 DAS (106 nmol C_2H_4 h^{-1} bag^{-1}) and then decreased to 36 nmoles C_2H_4 h^{-1} bag^{-1} at 105 DAS (Fig. 1).

For estimating the counts of diazotrophs, two randomly selected bags each, i.e., planted and unplanted, were selected each time after assay. Increase in nitrogenase activity of the soil-root system closely followed increase of diazotroph counts upto 68 days, particularly the diazotroph count of nonsterilized and sterilized root samples. Unlike the nitrogenase activity that decreased 68 DAS, the diazotroph count remained largely unchanged. Assay at 68 DAS gave both maximum nitrogenase activity (129 nmol C_2H_4 h^{-1} bag^{-1}) as well as maximum diazotroph count of sterilized (4×10^9 g^{-1} root) and nonsterilized (2×10^{11} g^{-1} root) macerate.

In studies with cultivar Indaf 5, the diazotroph counts were in general higher during the reproductive stage than during the vegetative stage. Root-adhering soil gave the maximum diazotroph count (8.5×10^9) g^{-1} dry root, followed by nonsterilized root macerate (3.4×10^9), and root wash (3.1×10^9). Surface-sterilization of root reduced the count from 3.4×10^9 to 1.6×10^8 (g root mass)$^{-1}$ for 5 min sterilization, and 1.3×10^6 (g root mass)$^{-1}$ for 60 min sterilization. The number of colony types of diazotrophs did not increase from the vegetative to repro-

Table 2. Screening of pure-line and hybrid selections of different origin for the root-associated nitrogenase activity.

Origin	Pedigree	No. of cultivars tested	No. of cultivars having nitrogenase activity (nmol C_2H_4 $plant^{-1}$ h^{-1}) >300	200-300	100-200	<100	Most active cultivar	Highest nitrogenase activity (nmol C_2H_4 $plant^{-1}$ h^{-1})
Karnataka	Hybrid	15	5	4	2	4	Indaf 9	361
Tamil Nadu	Hybrid	6	3	0	3	0	TNAU 169	488
Andhra Pradesh	Pure line	7	0	1	3	3	PR 1091	250
Madhya Pradesh	Pure line	5	1	1	2	1	JNR 852	354
Uttar Pradesh	Pure line	11	1	2	6	2	PES 83-2	324
Bihar	Pure line	4	0	0	2	2	RAU 5	183
Orissa	Pure line	2	1	0	1		B 7-7-43	314
Exotic collection	Pure line	3	1	0	1	1	IE 1012	403
Total		53	12 (23)[1]	8 (15)	20 (37)	13 (25)		

1. Figures in parentheses are percentages.

ductive growth stage. A maximum of 15 colony types were observed in nonrhizospheric soil, and surface-sterilized root macerate showed 5 to 6 colony types. A total of 40 isolates that grew consistently on N-free medium were finally obtained in pure cultures.

When the 40 diazotroph isolates from cultivar Indaf 5 were screened for ARA, all but 3 isolates

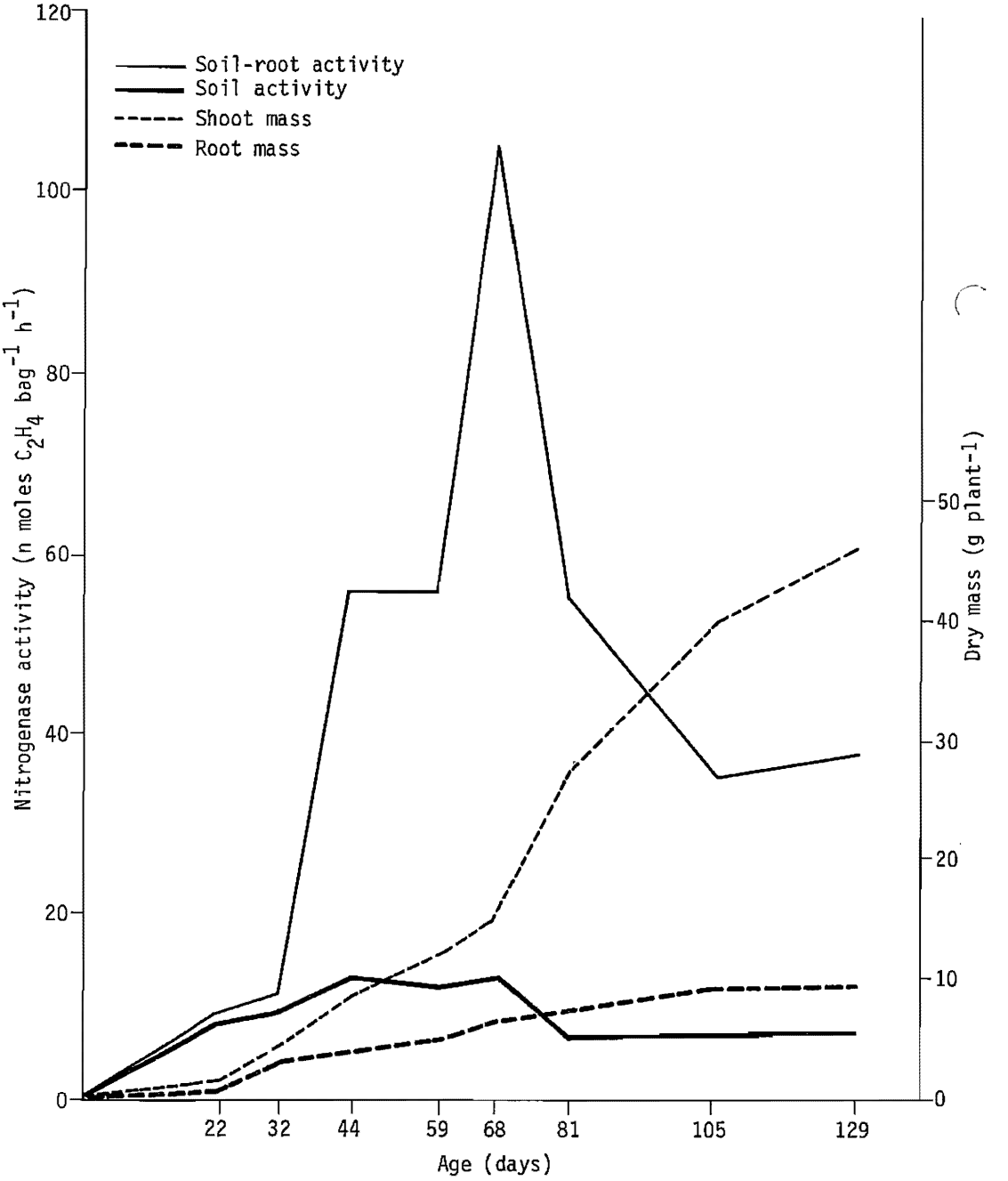

Figure 1. Seasonal pattern of nitrogenase activity and shoot and root mass of finger millet cultivar Indaf 5.

produced less than 2 nmoles C_2H_4 h^{-1} $tube^{-1}$. Of these three isolates, No. 38 and 45 were identified as *Enterobacter cloacae* and Isolate No. 23 was *Azospirillum*-like. The rest of the isolates were species of *Pseudomonas* or *Enterobacter cloacae*. Isolates from finger millet were compared with three reference cultures, i.e., NBRE (napier bajra root extract), *A. lipoferum* (4ABL), and *Azospirillum lipoferum* (ICM 1001) for *in-vitro* activity. The cultures NBRE and 4ABL showed four-fold more activity than the isolates from finger millet. However, *E. cloacae* showed higher activity than *A. lipoferum*. Highest activity (115 nmoles C_2H_4 h^{-1} $tube^{-1}$) was recorded in the NBRE culture.

Effect of Soil Moisture

Effect of moisture on nitrogenase activity of finger millet Indaf 5 was studied by manipulating the moisture regime in the soil and by using an intact-plant assay method (Wani et al. 1984). The plants were grown in soil in plastic bags, and a basal dose of 25 kg N and 50 kg each of P_2O_5 and K_2O ha^{-1} through urea, superphosphate, and potassium chloride was applied. Till the moisture-level treatments were imposed, all the plants were watered uniformly. Nitrogenase activity of the soil-root system as affected by six moisture levels (20% WHC to 90% WHC) increased with increasing soil moisture. There was no significant change in nitrogenase activity between moisture levels varying from 19% to 47% WHC. Linear regression of average nitrogenase activity against soil-moisture content indicated a significant correlation (r=0.788).

Diurnal Variation

Ten plants of cultivar Indaf 5 were grown in 6-L plastic pots containing 6 kg alluvial soil. A basal dose of 25 kg N and 50 kg each of P_2O_5 and K_2O was applied. The conditions for growing plants were similar to those described by Wani et al. (1984). Nitrogenase activity was estimated using intact-plant assay technique (Wani et al. 1984). Acetylene gas was injected at 1200 and gas samples were collected at 2 h intervals up to 2400. Soil temperature in the pot at each assay time was recorded. The diurnal pattern of nitrogenase activity is shown in Figure 2. Maximum activity of 104 nmol C_2H_4 h^{-1} $plant^{-1}$ was recorded between 10 am and 12 noon.

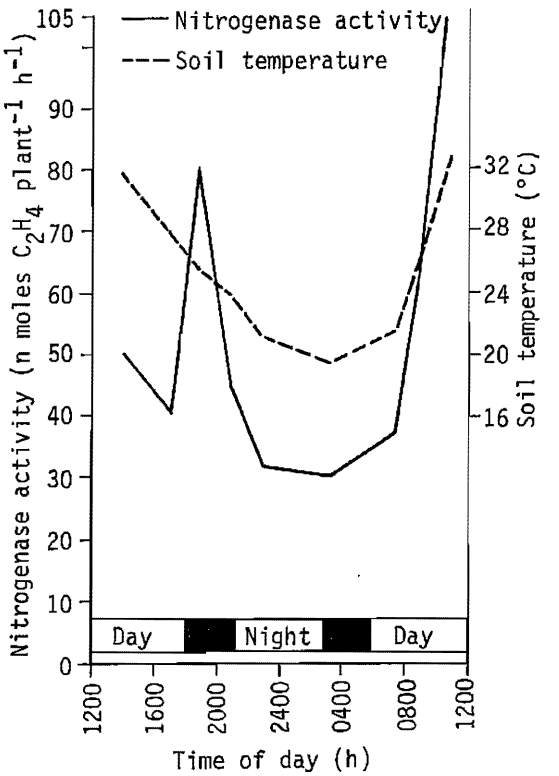

Figure 2. Diurnal pattern of nitrogenase activity of an intact-plant system of finger millet cultivar Indaf 5.

Response to Inoculation with Nitrogen-Fixing Bacteria

Response of finger millet cultivar Indaf 7 to seed inoculation with diazotroph cultures (obtained from ICRISAT Center) was studied at 25 and 50 kg ha^{-1} levels of applied nitrogen, under greenhouse and field situations. In the greenhouse, plants were raised in 25 cm × 15 cm iron cores filled with 3.75 kg alluvial soil. The basal dose consisted of a part of the N along with 50 kg each of P_2O_5 and K_2O. The remaining N was applied as a top dressing. In each case, a single plant was grown. The seeds were soaked for 18 h in broth culture of bacteria as per the treatment and dried. At sowing, each core was inoculated with 8 mL broth culture. The noninoculated broth served as the control. Surface soil in the cores was covered with gravel and the moisture content in the cores was maintained at around 70% WHC.

The grain and straw yields increased significantly

over the control due to inoculation with NBRE (37%) and SRI (35%) (Fig. 3). Inoculation with *A. lipoferum* (4 ABL) increased leaf number but not plant height over the control. Nitrogen alone or interaction of N × inoculation had no significant effect on yield parameters. Nitrogen content of grain increased 50% from inoculation with NBRE and 47% with SRI; total uptake of nitrogen by the plant increased 72% with NBRE and 66% with SRI. Inoculation had no effect on nitrogen content of straw. Interaction of N × inoculation (NBRE) increased N content of grain by 1%, while N alone had no effect.

In the field experiment, all the cultures used in the pot trial except the SRI isolate were used. The experiment was conducted following randomized-block design and 2 m × 2 m plots for each treatment, replicated four times. The N, P, and K treatments were as in the case of the pot experiment. Seeds were soaked in peat-inoculant suspension for 18 h, air dried and sown in rows 22.5 cm apart. Each plot was inoculated at sowing with peat-inoculant suspension (1 g peat inoculant suspended in 500 mL water) uniformly. Inoculation of finger millet with all the cultures of N_2-fixing bacteria increased grain or straw yields by 8-10% over the control plants, but the increase was not significant (Fig. 4). Application of N also gave nonsignificant yield increases over the control plants. Highest above-ground yield (5552 kg ha^{-1}) was obtained with *A. lipoferum* (ICM 1001) inoculation + 50 kg N ha^{-1}, followed by NBRE culture + 25 kg N ha^{-1} (5168 kg ha^{-1}). Nitrogen content of finger millet straw increased by 0.3% due to inoculation with NBRE and N uptake increased by 12 kg ha^{-1} (Fig. 5). Application of 50 kg N ha^{-1} increased grain nitrogen uptake by 25 kg ha^{-1}, but had no effect on straw nitrogen content.

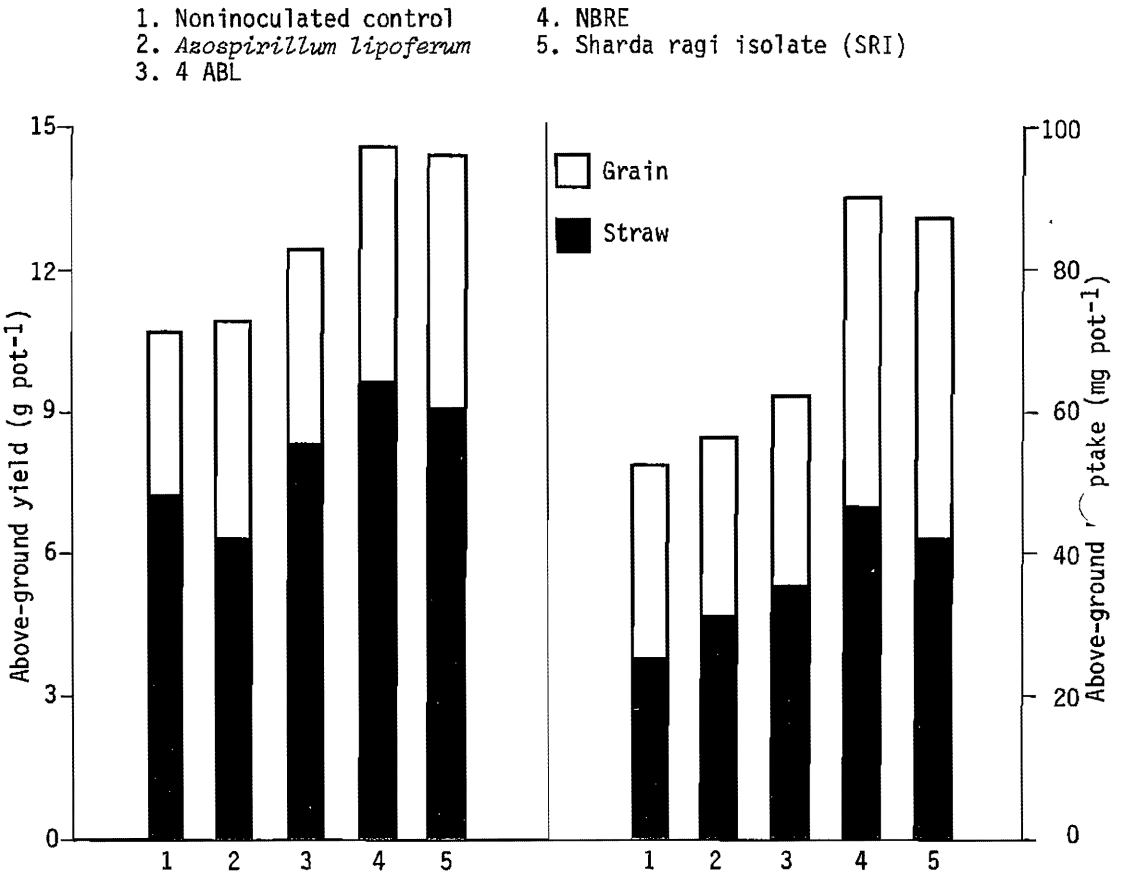

Figure 3. Response of finger millet cultivar Indaf 7 to inoculation with diazotrophs in a pot trial.

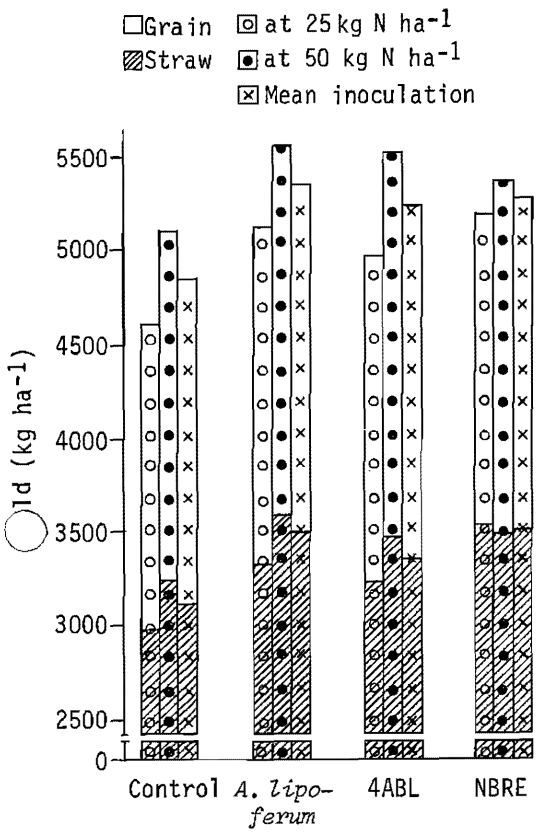

Figure 4. Response of field-grown finger millet cultivar Indaf 7 with nitrogen levels to inoculation with diazotrophs.

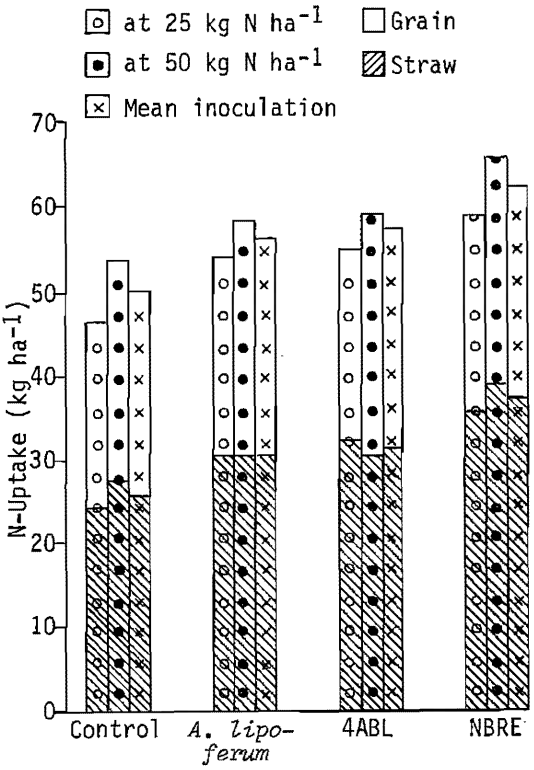

Figure 5. Nitrogen uptake by field-grown finger millet cultivar Indaf 7 as influenced by nitrogen levels and inoculations with diazotrophs.

Nitrogen Balance

Nitrogen balances (postharvest N-presowing N in) due to the growth of finger millet Indaf 5 as affected by planting, inoculation, and three levels of nitrogen (0, 25, and 50 kg ha^{-1}) were studied in pot culture in the greenhouse (Fig. 6). Maximum positive nitrogen balance (200 mg pot^{-1}) was found in inoculated finger millet at the 0 level applied-N, which also showed the least loss of soil nitrogen (5 mg pot^{-1}). Maximum loss of soil nitrogen was found in the unplanted pot with 50 kg ha^{-1} applied N. Final N-balances in inoculated and planted systems were 200, 168, and 198 mg pot^{-1}. In the unplanted system, there were negative balances of 11, 58, and 93 mg pot^{-1} respectively at the 0, 25, and 50 kg applied-N levels. Even though inoculated pots showed a higher balance (188 mg pot^{-1}) than the noninoculated pot (161 mg pot^{-1}), the difference was not significant. Planting Indaf 5 resulted in an N gain of 175 mg pot^{-1}, whereas in the unplanted system there was a loss of 49 mg N pot^{-1}. The system gains of N in pots due to inoculation extrapolated to a net gain of 6.3, 7.6, and 9.4 kg N ha^{-1}, respectively, at the 0, 25, and 50 kg ha^{-1} applied-N levels.

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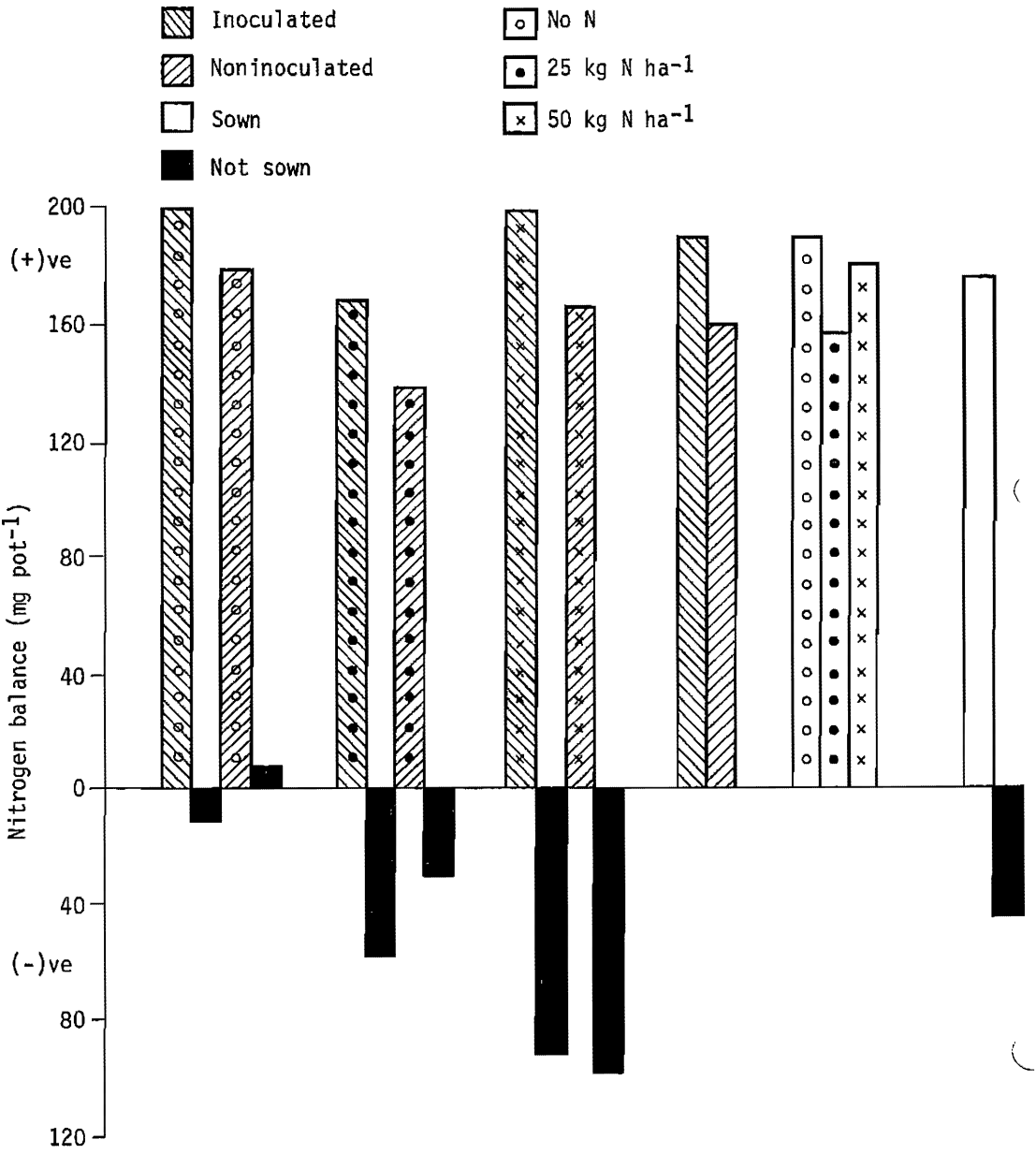

Figure 6. Nitrogen balance in pot-grown finger millet cultivar Indaf 5 as affected by nitrogen and inoculation (95-day-old plants).

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